ABSTRACT

1. INTRODUCTION

Diabetic nephropathy (DN) is a major complication of diabetes and the leading cause of end-stage renal disease (ESRD) and cardiovascular deaths, typically arising years after diabetes onset. The development and progression of DN are influenced not only by high blood glucose but also by various metabolic pathways, hemodynamic factors, and excessive production of reactive oxygen species (ROS). Natural products, originating from plants and other natural sources, are receiving increasing interest as alternative treatments for DN. Studies have shown that certain natural compounds can lower blood glucose and lipid levels and provide antioxidant benefits, which could help manage diabetes-related complications.

Natural compounds like **curcumin, catechin,** and extracts of ***Pueraria tuberosa*** and ***Pterocarpus santalinus*** offer antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, antiproliferative, proapoptotic, nephroprotective and anti-atherosclerotic effects. Despite their potential against diabetes, clinical use is limited by low bioavailability, poor solubility, and chemical instability.

Nanotechnology-based drug delivery systems enable precise, controlled drug release to diseased cells, enhancing concentration in target tissues and reducing systemic side effects by optimizing pharmacokinetics and biodistribution. This approach improves drug absorption, bioavailability, and stability, addressing limitations of traditional methods. Common nanocarriers include polymeric nanoparticles, lipid carriers, and liposomes.

2. OBJECTIVES

1. Formulate a carbohydrate polymer-based nanoformulation with curcumin (CUR) and epigallocatechin gallate (EGCG) and a nanostructured lipid carrier system with *Pueraria tuberosa* (PTE) and *Pterocarpus santalinus* (PSE), followed by physical characterization.
2. Establish a diabetic nephropathy model using STZ and confirm it through pancreatic and kidney histopathology.
3. Administer free and nanoformulated drugs to nephropathic animals for 60 days, assessing therapeutic efficacy via fasting blood glucose, serum creatinine, albumin, and blood urea nitrogen levels.

3. METHODOLOGY

Polymeric nanoparticles (PNPs) were prepared using the anti-solvent precipitation method. This included shellac and locust bean gum coacervated nanoparticles loaded with the combined drugs, CUR and EGCG (CESL-NP), along with single-drug nanoparticles containing only CUR (CSL-NP) and only EGCG (ESL-NP).

Nanostructured lipid carriers (NLCs) were prepared using the hot homogenization method. This included carriers loaded with combined plant extracts [(PTE+PSE)-NLC], along with single-extract carriers containing only PTE (PTE-NLC) and only PSE (PSE-NLC).

New RP-HPLC methods were developed for the simultaneous estimation of CUR and EGCG within CESL-NP, and PTE and PSE within (PTE+PSE)-NLC. The developed methods were validated according to the International Council for Harmonization (ICH) guidelines for system suitability criteria, followed by the determination of drug loading (%) and entrapment efficiency (%) for CESL-NP and (PTE+PSE)-NLC.

Characterization of the formulated nanoparticles was performed using various analytical techniques, including dynamic light scattering (DLS), fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), atomic force microscopy (AFM), high-resolution transmission electron microscopy (HRTEM), and field emission scanning electron microscopy (FESEM).

The solubility of free CUR and CESL-NP was evaluated, and the stability of CESL-NP and (PTE+PSE)-NLC were evaluated based on mean particle size, polydispersity index (PDI), zeta potential, and drug loading, after 90 days of storage. *In vitro* drug release studies for CESL-NP and (PTE+PSE)-NLC were conducted in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4).

In vivo studies involved developing a diabetic nephropathy animal model using a single STZ injection at 80 mg/kg body weight. Two weeks later, treatments with free drugs/extracts (CUR, EGCG, PTE, and PSE) and nanoformulated drugs/extracts [CSL-NP, ESL-NP, CESL-NP, PTE-NLC, PSE-NLC, and (PTE+PSE)-NLC] began. Each group received daily doses via oral gavage for 60 days, with fasting blood glucose levels measured weekly using an ACCU-CHEK Guide glucometer. The antidiabetic potential was evaluated by measuring fasting blood glucose and calculating percentage inhibition, while nephroprotective effects were assessed through kidney size, weight, kidney hypertrophy index (KHI), serum creatinine (Scr), albumin (Alb),

and blood urea nitrogen levels (BUN). Histological examinations were performed on thin sections of pancreatic and kidney tissues from untreated mice and those treated with free and nanoformulated drugs.

Statistical analysis employed one-way analysis of variance (ANOVA), followed by Dunnett's t-test to identify specific differences between experimental and control groups.

4. RESULTS AND DISCUSSION

The CESL-NP demonstrated excellent drug loading, with 16.69% for CUR and 17.56% for EGCG, and entrapment efficiencies of 95.2% for CUR and 94.35% for EGCG. The (PTE+PSE)-NLC formulation exhibited a loading capacity of 10.1% for PTE and 9.92% for PSE, with entrapment efficiencies of 91.74% and 92.47%, respectively.

Morphological characterization showed that both nanoformulations, CESL-NP and (PTE+PSE)-NLC, were spherical, uniformly dispersed, and within the nanoscale range with no aggregation. Physicochemical analysis confirmed successful encapsulation of the drugs (CUR and EGCG) and extracts (PTE and PSE) into the nanoparticle matrices, resulting in a conversion from crystalline to amorphous structure. This transformation is known to enhance the solubility of the free drug or extract when encapsulated.

Solubility studies revealed a 70-fold increase in the water solubility of CUR when encapsulated within CESL-NP compared to free CUR, attributed to the protective effects of the nanoparticle matrix against aggregation. After storage of CESL-NP and (PTE+PSE)-NLC at 4°C for 90 days, only minimal changes were observed in particle size and PDI, along with a slight decrease in zeta potential and drug loading. Stability studies indicated that the formulated nanoparticles maintained their stability without significant changes in the measured parameters over the 90-day period.

In vitro release studies demonstrated an initial burst release of CUR and EGCG from CESL-NP, as well as PTE and PSE from (PTE+PSE)-NLC, within the first 12 hours, followed by a sustained release over seven days in SGF and SIF media. The rapid initial release is likely due to the diffusion of loosely bound drugs and extracts, while the sustained release results from their incorporation within the nanoparticle matrices. Notably, CUR and EGCG from CESL-

NP, along with PTE and PSE from (PTE+PSE)-NLC, were released more rapidly in SIF (pH 7.4) than in SGF (pH 1.2).

In vivo antidiabetic studies demonstrated that CESL-NP and (PTE+PSE)-NLC reduced elevated blood glucose levels by 95.6% and 96.5%, respectively, after eight weeks. These reductions significantly outperformed those of the single-drug/extract nanoformulations (CSL-NP, ESL-NP, PTE-NLC, and PSE-NLC), as well as the free drugs (36.14%) and free extracts (15.4%). CESL-NP provided a 38.68-fold greater inhibition of blood glucose elevation, while (PTE+PSE)-NLC demonstrated a 31.28-fold greater inhibition compared to the free drugs and extracts. These findings suggest that CESL-NP and (PTE+PSE)-NLC have superior blood glucose-lowering capabilities, likely due to synergistic effects and enhanced bioavailability.

In vivo antinephritic studies demonstrated that CESL-NP and (PTE+PSE)-NLC significantly reduced the increased kidney size, weight, KHI, Scr, and BUN levels, while preserving normal body weight and Alb levels, indicating improved renal function. In contrast, the untreated diabetic group exhibited kidney enlargement due to inflammation, a hallmark of early DN.

Histopathological analysis of pancreatic and kidney tissue sections further supported the therapeutic efficacy of CESL-NP and (PTE+PSE)-NLC. These formulations exhibited regenerative effects, as indicated by an increase in healthy pancreatic β-cells and kidney podocytes, along with a reduction in pathological features in treated groups compared to the untreated group.

Signature of Candidate:

Gyancho Tshering Bhutia

Signature of Supervisor(s):

1. 05/11/24

Tanmoy Bera, M. Pharm., Ph.D.
Professor
Division of Medicinal Biochemistry
Department of Pharmaceutical Tech.
Jadavpur University, Kolkata - 700 032

2. 05/11/2024

Dr. Manas Bhowmik
Associate Professor
Dept. of Pharmaceutical Technology
Jadavpur University, Kol-700 032
West Bengal, India